

## Evaluation of the antimicrobial activity of endodontic sealers against lactobacilli: An *in vitro* study

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### ABSTRACT

**Aim:** This study is done to find out the antimicrobial effect of sealers used in endodontic treatment against lactobacilli which is the major bacterial flora in patients with poor oral hygiene. The sealers which have good antimicrobial property prevent recolonization of the bacteria in the tooth cavity. **Background:** Microleakage is a common issue in endodontic treatment. This leads to the recolonization of the pulpal tissue by the salivary flora. An effective sealer that prevents the entry of salivary flora will be beneficial for a successful endodontic treatment. Apart from the sealing ability, the antimicrobial activity of the sealer will definitely reduce the microbial recolonization. **Materials and Methods:** An *in vitro* study is conducted to test the antimicrobial activity of the sealants. Agar well diffusion method is used in this study. The sealants are filled in with the cavity and the organism is spread on the agar surface. After incubation, the zone of inhibition is noted around the well and compared with different types of sealants. **Results:** The zone of inhibition produced by the three compounds tested is tabulated. It shows the mean zone at inhibition shown by the three compounds tested against lactobacilli. Among the three compounds tested the pit and fissure sealer has shown the maximum zone of inhibition. The other two sealants tested are found to be not effective against lactobacilli. **Conclusion:** Pit and fissure sealers have better antimicrobial property when compared to composite and GIC. Pit and fissure sealers have lesser susceptibility to bacteria and prevents recolonisation of bacteria.

**KEY WORDS:** Anti-microbial effects, Lactobacilli, Pit and fissure sealers, Secondary caries

### INTRODUCTION

Root canal treatment, also known as endodontic therapy, endodontic treatment, root canal therapy, or simply root canal is a treatment sequence for the infected tooth which results in the elimination of infection and the protection of the decontaminated tooth from future microbial invasion.<sup>[1]</sup>

Causes of endodontic failure are a persistent microbiological invasion. The role of bacteria in peri-radicular infection has been well established in literature, and endodontic treatment will be afflicted with a higher chance of failure if microorganisms persist in the canals at the time of root canal obturation.<sup>[1]</sup> A leaky apical seal is also a contributory factor in endodontic failure due to microbiological persistence. Seepage of acids is likely to occur if apical

seal is not properly established. This can perpetuate peri-radicular inflammation anytime.

Almost 700 bacterial species can be found in the oral cavity, with any particular individual harboring 100–200 of these species.<sup>[2]</sup> The main bacterium that causes the formation of the infection is lactobacilli, *Streptococcus mutans*, *Staphylococcus*, etc. Some microorganisms are resistant to antimicrobial treatment and can survive in the root canal after biomechanical preparation. In infected root-filled teeth, any medicament remnants and root filling material are additional factors to influence pathogenicity.<sup>[2]</sup>

Fluoride, the pivot of preventive dentistry, continues to be the cornerstone of caries prevention programs. The success of fluoride in caries prevention of smooth surfaces has made dental caries primarily a disease of pits and fissures of teeth. Occlusal pits and fissures are the most susceptible sites for the development of dental caries, and they occur in areas where prevention is difficult.<sup>[3]</sup> Although only 12.5% of all

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the teeth surfaces are occlusal, these surfaces develop more than two-thirds of total caries in children.<sup>[4]</sup>

Pit and fissure sealants were introduced in 1967, and their effectiveness was recognized by the American Dental Association in 1971.<sup>[4]</sup> The susceptibility of the occlusal surfaces of the permanent molars to dental caries has increased the applicability of pit and fissure sealants.

The clinical diagnosis of incipient carious lesions in posterior teeth is complicated by the depth, narrowness, and complexity of the pits and fissures. Consequently, such lesions may remain undetected and inadvertently be sealed with a dental sealant.<sup>[5]</sup> The fate of the bacteria is of significance then.

Additional protection could be afforded against any subsequent deterioration of the bond at the resin-tooth interface if the sealant possesses some antibacterial property.<sup>[5]</sup> Since *S. mutans* and *Lactobacillus acidophilus* are associated with the initiation of dental caries and the progression of the established lesion, respectively, a reduction in the number of these bacteria in the resin-tooth interface would be expected to influence the caries incidence.<sup>[6]</sup>

This study was performed to assess the antibacterial properties of pit and fissure sealants when compared with the antibacterial properties of fluoride-releasing and nonfluoride-pit and fissure sealants.

## MATERIALS AND METHODS

It is an *in vitro* study. Three types of restorative sealants were used against *Lactobacillus*. The sealants used were pit and fissure sealants, glass-ionomer cement, and composite. These materials are widely used in the tooth restorations, and the success rate is higher in the restorations.

A standard strain of lactobacilli is used for the study. Bacterial suspension of turbidity matching 0.5 McFarland standard is prepared from the overnight culture of the lactobacilli grown on MacConkey Agar. Lactobacilli species is the most predominant bacterial strain present in the oral cavity. Its proportion is increased in patients with poor oral hygiene. It is widely isolated in all clinical specimen collected from the mouth. It plays a key role by facilitating the opportunistic pathogens in oral diseases. The acidogenic nature of *Lactobacillus* sp. is very

**Table 1: Diameter of the zone of inhibition of the sealants against lactobacilli**

Sealant used	Mean of the zone of inhibition
Composite	0
Glass-ionomer cement	0
Pit and fissure sealant	21.8 mm

important in hard tissue lesions. It is present in the plaque in patients with compromised oral hygiene. It is one the organisms that are implicated as a reason in failed root canal. Presence of lactobacilli at the interface of restoration will promote leakage and ultimately resulting in failure of restoration.

The medium for testing the antimicrobial activity is Mueller-Hinton Agar (HiMedia). The experiment is conducted by agar-well diffusion method. A thick layer of Mueller-Hinton Agar is poured on to the 10 mm disposable Petri dish. Wells were cut after the agar is set. The well is cut with 6 mm sterile stainless steel tube. Thirty wells were cut and 10 wells were allotted for each material tested. The bacteria are spread on the surface of the agar using a sterile swab. Composite and pit and fissure sealants are filled in the wells using a sterile probe. The base and catalyst component of glass ionomer cement are mixed using a sterile agates spatula in a sterile paper slab and is filled into the agar well. Enough material is filled in the wells so that it comes in contact with the boundaries of the well and is filled up to the surface. These plates are incubated at 37°C for 24 h aerobically and are checked for the zone of inhibition. The zone of inhibition is measured in mm using a scale. The zone of inhibition determines the antimicrobial property of the sealants used. Depending on the diameter of the zone, the conclusion for the best antimicrobial property among the sealants used is evaluated.

## RESULTS

The zone of inhibition was tested by agar well diffusion method. The pit and fissure sealants showed significant antimicrobial property by giving the larger zone of inhibition, whereas the other two components glass-ionomer cement and composite do not exhibit zone of inhibition thus resulting in no antimicrobial property [Table 1].

## DISCUSSION

The experiment was conducted by agar well diffusion method with 10 replicates in each group of the sealants tested against lactobacilli. Composite, glass-ionomer cement, and pit and fissure sealant were added to the wells, respectively. These plates were incubated at 37°C for 24 h aerobically and are checked for the zone of inhibition. The well which has a significant zone of inhibition has the highest antimicrobial activity.

This study is done to assess the antimicrobial property of the sealers used in endodontic treatment. Even though the host is unable to eliminate the root canal infection, mobilization and further concentration of defense components at the peri-radicular tissues impeding the spread of infections.<sup>[7]</sup> Thus, the applications prevent the infection of bacterium in the

root canal to cause secondary infections. However, due to improper application of sealants during root canal treatment there is cause of secondary infections leading to formation of caries in the root canal. The cariogenic bacteria in the saliva will grow when the environment of micro cracks is appropriate, thereby producing secondary caries.<sup>[8]</sup>

The clinical diagnosis of recurrent caries is the most common reason for replacement of all types of restorations in general dental practice. Marked variations in the diagnosis of the lesions have been reported. The prevention of recurrent lesions by the use of fluoride-releasing restorative materials has not been successful. They are repaired by the complete replacement of the restoration.<sup>[9]</sup> The presence of zinc phosphate in the pit and fissure sealants is the main reason for the antimicrobial property of the pit and fissure sealants.

From the experiment done, glass-ionomer cement and composite did not give any zone on incubation. This concludes that pit and fissure sealants have high antimicrobial property as they formed a mean zone of 22.3 mm.

The clinical effectiveness of pit and fissure sealants in preventing occlusal caries has been well-documented, but there is some concern about bacteria left beneath sealants. The sealing of pits and fissures resulted in a reduction of 98% of viable bacteria in carious lesions by seal bacteria from the nutrient supply.<sup>[6]</sup> This is mainly because of the fluoride release from pit and fissure sealants onto the walls of the tooth cavity.

Most clinical studies of pit and fissure sealants agree that a positive relationship exists between the sealant retention and the caries protection benefits, both in preventing and inhibiting incipient caries in enamel and dentin.<sup>[10]</sup> Sealants are often used to occlude at-risk pits and fissures on teeth. When properly placed, sealants provide a physical barrier between the dental enamel and the oral environment shielding the tooth surface from an acid challenge. Sealants are effective in arresting caries progression when properly applied to incipient demineralized lesions. Thus fluoride releasing sealants are really valuable on the market.<sup>[11]</sup>

The treatment and prevention of secondary caries formation are done by maintaining proper oral hygiene and checked from progressing by timely intervention. Patient education and motivation are crucial to this. Routine dental check-up and meticulous oral hygiene practices can prevent the cavitation of this lesion and negate the oral health burden of the patient.<sup>[12]</sup> Exposure to low concentration of fluoride produces the optimal remineralization environment. Fluoride is typically applied as toothpastes, gels, varnishes,

foam, and mouth rinses. Periodic, professionally-applied topical fluoride agents can also be beneficial. The American Dental Association has approved the use of 1.23% acidulated phosphate fluoride (APF) gel/foam, 8% stannous fluoride solution, and 2% sodium fluoride gel as professionally applied topical agents. European studies of a 5% sodium fluoride varnish have also demonstrated caries preventive benefits similar to APF gel/foam when applied topically.<sup>[13]</sup>

## CONCLUSION

This study was done to test the antimicrobial activity of sealers on the progression of incipient caries due to failure of endodontic treatment. Thus, pit and fissure sealants have found to have more antimicrobial property having lesser susceptibility to recolonization of the bacteria and failure of treatment. In this study, we used lactobacilli as an indicator to check the antimicrobial activity. Radiating lactobacilli on the preparation site is more reasonable compared with less acidogenic bacterial strains because acid is the main reason for the leakage, detachment, and failure of the restorative materials. The antimicrobial activity of the pit and fissure sealant is found to be more specific to the predominant bacterial flora in the mouth which is commonly associated with complications and treatment failures. Thus, according to this study, pit and fissure sealants are found to be the best choice.

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